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Plasma Opioid Peptide Responses During Heat Acclimation in Humans¹

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KRAEMER, W. J., L. E. ARMSTRONG, L. J. MARCHITELLI, R. W. HUBBARD AND N. LEVA. Plasma opioid peptide responses during heat acclimation in humans. PEPTIDES 8(4) 715-719, 1987.—Plasma β -endorphin, Met-enkephalin and Peptide F immunoreactivity (ir) were measured at rest and following exercise on three days (days 1, 4, 8) of an eight day heat acclimation regime. Fourteen male subjects demonstrated physiological heat acclimation adaptations. Our data demonstrated a differential response of peripheral plasma levels of endogenous opioid peptides (EOP) to exercise in the heat. In addition, EOP did not follow the same time-course of other physiological adaptations as no differences (day 1 vs. 4 vs. 8) in resting or exercise levels were observed over the eight day heat acclimation regime. Significant increases in β -endorphin ir (pre- to post-exercise) appear to reflect concomitant exercise-heat related changes. The increased peripheral levels of β -endorphin were correlated to plasma levels of cortisol. Heat and exercise stress may result in a reduction of Met-enkephalin ir observed in peripheral plasma and might be due to degradation or a decrease in processing from the larger precursors. The differential responses of EOP suggest the possibility of separate physiological roles for these peptides during exercise in the heat but peripheral plasma levels of EOP do not appear to reflect acute heat acclimation changes.

β -Endorphin Met-enkephalin Peptide F Cortisol Heat acclimation

ENDOGENOUS opioid peptides (EOP) appear to be involved with thermoregulation and adaptation to extremes in the thermal environment [3,14]. Studies using the opiate antagonist naloxone which examined hypothermic and hyperthermic actions suggest that the enkephalins and β -endorphin may be working through separate opiate receptors [2, 5, 10, 14]. Pharmacological studies have also supported possible roles for EOP in thermoregulation [6, 23, 24]. To date, the results of most studies in which opioid peptides were administered indicate that these peptides can act centrally to influence thermoregulation. The possibility of peripheral thermoregulatory effects have been considered [25]. Increases in rectal temperature (Tre) from combined exercise and heat stress have been associated with increases in peripheral circulating plasma concentrations of β -endorphin/ β -lipotropin in humans [16]. While these data all support a possible role for EOP in acute thermoregulation, no data are available concerning the responses of EOP in humans to repeated heat stress exposures.

Most heat acclimation adaptations, induced by exercise and artificial climatic conditions in an environmental chamber, have been shown to occur over a 3 to 8 day period [1]. Heat acclimation can be achieved by exercising in moderate to hot ambient conditions for 1 to 2 hr daily [21]. The commonly observed physiological adaptations to heat stress

over repeated exposures include: a decreased exercising core temperature, a decreased exercising heart rate and a plasma volume expansion [1].

It has been firmly established that exercise can significantly perturb thermoregulatory mechanisms. In addition, exercise previously has been demonstrated to increase various EOP levels such as methionine-enkephalin (Met-enkephalin), Peptide F and β -endorphin [4, 7, 9, 11, 12, 15, 19]. It has been suggested that training may also affect exercise responses of β -endorphin and Peptide F [4,19]. The purpose of this study was to examine the resting and exercise plasma responses of β -endorphin, Met-enkephalin, and Peptide F (preproenkephalin 107-140; 33 amino acids) to an eight day heat acclimation regime in order to: (1) determine if any changes in resting or exercise EOP levels occur with heat acclimation, (2) document any differential responses of different EOP, and (3) gain insights into possible explanations for observed plasma levels of EOP.

METHOD

Fourteen healthy male subjects volunteered as subjects for this study. The physical characteristics of the subjects were mean \pm SD: age (years) 28.4 \pm 1.9, height 177.0 \pm 2.0 (cm), weight 79.77 \pm 3.78 (kg), % body fat 18.7 \pm 1.4, maximal

¹The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as an official department of the Army position, policy or decision, unless so designated by other official documentation. Human subjects participated in this study after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 on Use of Volunteers in Research.

oxygen consumption 45.74 ± 1.96 (ml·kg·min⁻¹) and surface area 1.96 ± 0.50 (m²). After informed consent was obtained, all subjects completed a health questionnaire, activity questionnaire and history of heat exposure prior to testing. Data from these forms were examined to determine if each subject was unacclimatized prior to the study.

One day prior to the eight day heat acclimation regime, each subject was given a progressive exercise test to determine maximum oxygen consumption ($\text{VO}_{2\text{max}}$), which was used to calculate the relative exercise intensities of the exercise training. During testing and training a semi-automated system was used to collect and analyze expired gases. This system consisted of a Hewlett-Packard 85B computer, scanner, and digital voltmeter and were interfaced with a gas meter (Parkinson-Cowan), oxygen analyzer (Applied Electrochemistry S3A) and carbon dioxide analyzer (Beckman LB2). P_{re} was monitored every four min using a rectal probe (inserted 8 cm beyond the anal sphincter).

The heat acclimation regime consisted of eight days of treadmill exercise (0% grade). Each exposure consisted of 50 min of intermittent exercise during 100 min in an environmental chamber maintained at $41.2 \pm 0.5^\circ\text{C}$, $39.0 \pm 1.7\%$ relative humidity (RH). Exercise protocols on days 1 and 8 were performed at the identical duration and intensity ($71.8 \pm 2.9\%$ $\text{VO}_{2\text{max}}$ for running). Day 4 running exercise was performed at the intensity of $67.6 \pm 2.3\%$ $\text{VO}_{2\text{max}}$. The other days consisted of similar high intensity interval exercise. Water was drunk ad lib throughout all trials, but could not be sprayed or poured on the body.

Subjects were encouraged to drink adequate water when they were not in the climatic chamber. Upon arriving at the testing site each day, subjects were weighed and produced a urine sample which was analyzed for specific gravity. If any subject had a urine specific gravity greater than 1.030, he drank water until a more dilute urine was produced (specific gravity < 1.030).

A 20 min standing equilibration period in the heat preceded collection of each antecubital blood sample (days 1, 4 and 8). A second antecubital blood sample was taken immediately post-exercise. Blood samples for measurement of plasma cortisol, Met-enkephalin and β -endorphin were collected into specialized chilled glass vacutainers containing the anticoagulant EDTA (7.2 mg/5 ml whole blood). Blood was mixed gently and immediately centrifuged for fifteen min at $760 \times g$, 4°C . Blood samples for measurement of plasma Peptide F were collected in chilled glass vacutainers containing sodium heparin and 25 $\mu\text{l}/\text{ml}$ whole blood of aprotinin (Sigma Chemical Co., St. Louis, MO), gently mixed and centrifuged at $1500 \times g$, 4°C for fifteen min. Plasma samples were stored at -115°C until analyzed. Hemoglobin was analyzed using the cyanemethomoglobin method (Hycel Inc., Houston, TX) and hematocrit was analyzed in triplicate using a micro-capillary technique. Serum sodium (Na^+) and potassium (K^+) were determined using a flame photometer (Rainin Instruments, FLM3). Changes in plasma volume ($\%\Delta\text{PV}$) were calculated from changes in hematocrit and hemoglobin [8].

Ten μl samples were used in duplicate to perform the radioimmunoassay (New England Nuclear, North Billerica, MA) for plasma cortisol immunoreactivity. Five hundred μl of tracer and antiserum complex were added to the sample tubes simultaneously by use of a Beckman Accu-prep 221 automatic dilutor. Tubes were vortexed and incubated at room temperature for 30 min. The tubes were then cen-

trifuged at $1500 \times g$ for 10 min at 4°C , the supernatant decanted and pellets were counted for radioactivity.

Prior to radioimmunoassay (Immuno Nuclear Corp., Stillwater, MN), Met-enkephalin was extracted from the plasma using ODS-silica columns (C_{18} cartridges, Waters Inc.). One ml of plasma was acidified with 100 μl of 1 M HCl and loaded on the column. The Met-enkephalin was then eluted off the column with a total of 4 ml of methanol. The methanol eluate was evaporated to dryness at 37°C . Samples were reconstituted with 1 ml of BSA-phosphate. Samples were vortexed and placed at 37°C for 10 min followed by another vortex. Two hundred μl aliquots were assayed in duplicate. The radioimmunoassay employed simultaneous addition of sample, rabbit anti-met-enkephalin antibody, and ^{125}I Met-enkephalin followed by an overnight incubation at 4°C . The plasma immunoreactivity showed parallel displacement to Met-enkephalin. Cross-reactivity of Met-enkephalin antibody was 2.8% with Leu-enkephalin and $< 0.002\%$ -substance P, $< 0.002\%$ - β -endorphin, 0.10% alpha endorphin, $< 0.002\%$ -porcine dynorphin¹⁻¹³, $< 0.002\%$ -alpha neo-endorphin.

Prior to radioimmunoassay (Immuno Nuclear Corp., Stillwater, MN), one ml of plasma was concentrated and β -endorphin was extracted using a column (chromatography column, fine filter, 15–45 microns, Evergreen Scientific) to mix plasma and β -endorphin antibody coated Sepharose particles. β -Endorphin was washed with saline and eluted from the column filter with a total of 0.5 ml 0.025 N HCl and 200 μl were immediately assayed in duplicate. The radioimmunoassay involved a disequilibrium method based on an antibody with high sensitivity to β -endorphin. The sample and first antibody were incubated for 20 hr at 4°C . ^{125}I β -endorphin was added followed by a second incubation for 20 hr at 4°C . Phase separation was completed in 20 min with a pre-precipitated complex of second antibody, carrier and PEG added in a single pipetting step. The plasma immunoreactivity showed parallel displacement to β -endorphin. Cross-reactivity of the β -endorphin antibody was 5% with β -lipotropin, 100%-Des-Tyr¹ human β -endorphin, 100%-2-Me-Ala² β -endorphin, 100%-N-acetyl β -endorphin, $< 0.01\%$ - β -LPH⁶¹⁻⁷⁷, $< 0.01\%$ -D-Ala²- β -endorphin, $< 0.01\%$ -dynorphin, $< 0.01\%$ -alpha neo-endorphin, $< 0.01\%$ -Leu-enkephalin, $< 0.01\%$ -Met-enkephalin, $< 0.01\%$ -ACTH¹⁻²⁴, $< 0.01\%$ -alpha MSH, $< 0.01\%$ -prolactin, $< 0.01\%$ -LH, $< 0.01\%$ -FSH, $< 0.01\%$ -TSH, $< 0.01\%$ -oxytocin.

In order to avoid non-specific displacement in the radioimmunoassay, the Peptide F from each sample was partially purified using "HPLC type minicolumns" (C_{18} extraction column, J. T. Baker Co.). The methods used to purify the samples and conduct the radioimmunoassay were previously described [19,20]. The mean percent recovery of radioactively labeled Peptide F with this procedure was 86%. Peptide F was measured by radioimmunoassay in duplicate using commercially available ^{125}I ligand and antisera (Peninsula Laboratories, Belmont, CA). The antisera showed the following cross-reactivities: 0.047%-Met-enkephalin, 0.046%-Leu-enkephalin, 0.041%-Neo-endorphin, 0.037%-Dynorphin¹⁻¹⁷ and 0.034%- β -endorphin. The plasma immunoreactivity showed parallel displacement to Peptide F.

All samples for each specific radioimmunoassay were measured in the same assay to avoid run to run assay variations. Determinations of the different plasma ir were accomplished with the use of a Beckman 5500 Gamma counter and data reduction system.

Statistical evaluation of the data was accomplished by

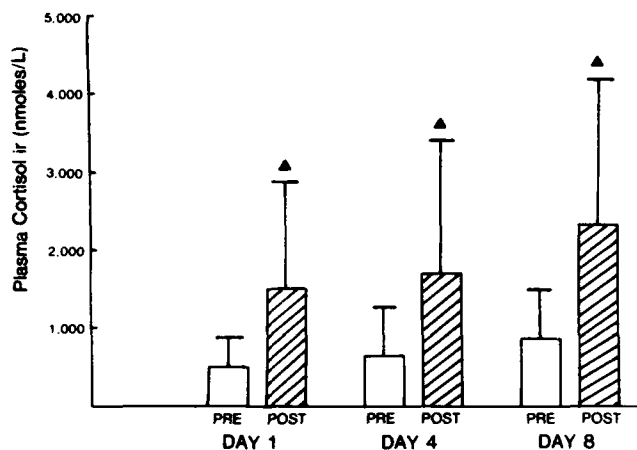


FIG. 1. Plasma cortisol immunoreactivity responses to eight days of heat acclimation. Δ Indicates a significant ($p < 0.05$) difference pre- to post-exercise.

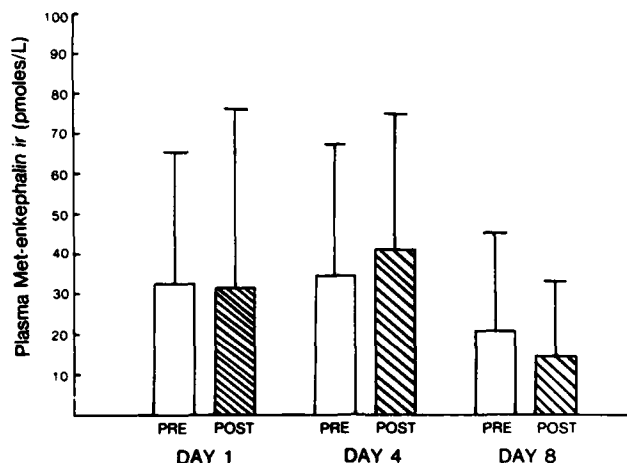


FIG. 2. Plasma Met-enkephalin immunoreactivity responses to eight days of heat acclimation.

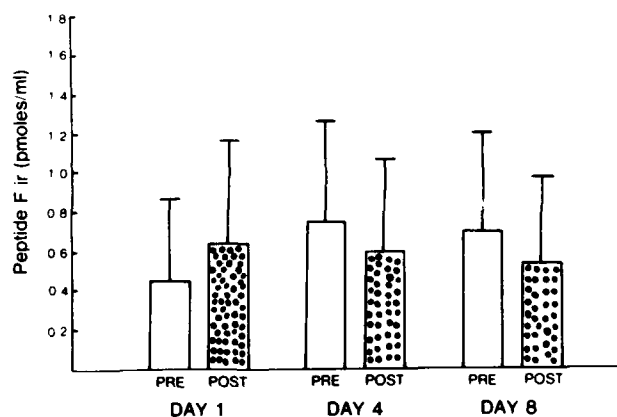


FIG. 3. Plasma Peptide F immunoreactivity responses to eight days of heat acclimation.

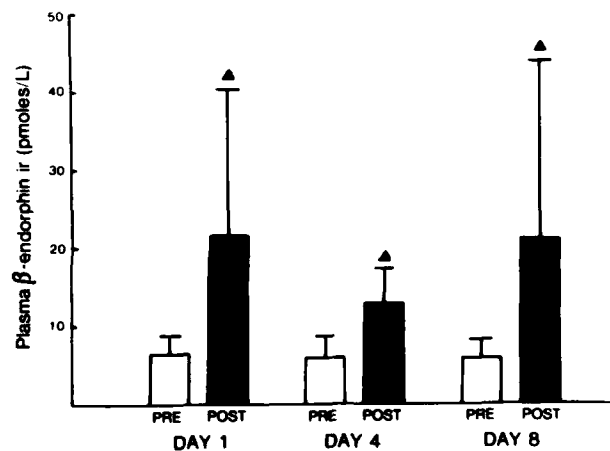


FIG. 4. Plasma β -endorphin immunoreactivity responses to eight days of heat acclimation. Δ Indicates a significant ($p < 0.05$) difference pre- to post-exercise.

using a 3×2 Analysis of Variance (ANOVA) (days \times pre/post). Subsequent post hoc analysis was performed using a Tukey test. Two-tailed paired t -tests were used to compare resting 25°C and hot levels of the EOP examined. Additionally, Pearson product moment correlation coefficients were calculated for the data set. In this study, significance was chosen as $p < 0.05$.

RESULTS

All of the subjects were unacclimatized at the beginning of this investigation. No significant differences were found when comparing mean entering body weight or mean entering urine specific gravity across the eight days. No significant differences were observed between the running intensities chosen on the three different days.

To examine the physiological effects of daily heat acclimation trials, a number of physiological variables were compared. The following variables all demonstrated significant decreases in mean values from day 1 to day 8; final exercise

heart rate (170 ± 3 vs. 144 ± 5 bpm), Δ heart rate (rest to end of exercise) (84 ± 3 vs. 68 ± 6 bpm), final Tre (39.17 ± 0.10 vs. $38.52 \pm 0.16^\circ\text{C}$) and Δ Tre (rest to end of exercise) (2.04 ± 0.09 vs. $1.46 \pm 0.16^\circ\text{C}$). Resting, pre-exercise plasma volume changes and expansion were compared from days 1 through 8. Plasma volume significantly expanded ($+5.9\%$) during the first four days of heat acclimation and stabilized through day 8 ($+5.2\%$). There was also a significantly better defense of plasma volume during exercise on day 8 than on day 1 (-5.1 ± 1.1 vs. $-7.1 \pm 0.9\%$).

No significant differences were found in serum sodium between day 1, 4 or 8 for pre- or post-exercise values (day 1, pre= 141 ± 1 , post= 140 ± 1 ; day 4, pre= 141 ± 1 , post= 140 ± 1 ; day 8, pre= 140 ± 1 , post= 141 ± 1 mmol/l). Serum potassium concentrations were significantly higher post-exercise on each of the three days, but no differences between days were observed (day 1, pre= 4.3 ± 0.1 , post= 4.7 ± 0.1 ; day 4, pre= 4.5 ± 0.1 , post= 4.7 ± 0.1 ; day 8, pre= 4.4 ± 0.1 , post= 4.8 ± 0.1 mmol/l).

There were significant increases in plasma cortisol ir pre-

to post-exercise on each day but there were no significant differences between days for resting or exercise values (Fig. 1). No significant differences were observed in Met-enkephalin ir and Peptide F ir (pre- to post-exercise in the heat) or between-days for resting or exercise values (Figs. 2 and 3). β -Endorphin significantly increased pre- to post-exercise on each day but no significant differences were observed between days (Fig. 4). A significant correlation ($r=0.45$) was observed between all post-exercise cortisol and post-exercise β -endorphin concentrations. The data set produced no other significant intercorrelations.

Although the primary purpose of this study was not to evaluate cool and hot responses, we randomly obtained blood from six of the test subjects in ambient conditions (25°C) to evaluate the possible effects of the intense heat on the resting plasma levels of the EOP measured. The comparative resting values for the six subjects were as follows (ambient/hot) (mean \pm SD): Peptide F [0.056 ± 0.021 pM/ml/ 0.444 ± 0.294 pM/ml, $p<0.05$], β -endorphin [4.48 ± 2.29 pM/l/ 5.18 ± 2.60 pM/l, $p>0.05$], Met-enkephalin [23.74 ± 18.47 pM/l/ 32.18 ± 15.28 pM/l, $p>0.05$]. Peptide F was the only EOP to be significantly elevated by the 20 min of intense heat exposure prior to exercise.

DISCUSSION

Comparison of the physiological measurements on days 1 and 8 (identical trials) indicated that typical heat acclimation adaptations (decreased exercising core temperature, a decreased exercising heart rate and plasma volume expansion) had occurred [1]. Despite these heat adaptations, no changes in resting or exercise responses of any EOP were observed over this period of time. In fact, the trend in the response was a tendency for the values to decline on day 8. Rather than speculate on a positive response during a longer heat acclimation period, the simplest explanation suggests that plasma levels of EOP do not respond to the acclimation process *per se*.

β -Endorphin ir significantly increased pre- to post-exercise on each of the three days examined. This is consistent with a response pattern keyed predominantly to exercise stress rather than thermoregulatory strain. Total heat stress is defined as the sum of the heat generated in the body (metabolic heat) plus the heat gained from the environment (environmental heat) minus the heat lost from the body to the environment. The body's response to total heat stress is defined as heat strain. Since both environmental and metabolic heat stress remained constant and yet rectal temperature declined, the heat loss mechanisms improved to effect a reduction in heat strain. Thus, across time ambient and metabolic stressors remained constant but thermoregulatory strain declined. The response pattern of β -endorphin, pre- to post-exercise, is therefore consistent with the metabolic demands of the exercise intensity.

The post-exercise plasma concentrations of β -endorphin observed in this study were higher than previous values reported for similar exercise intensities in thermoneutral conditions [4, 9, 12, 15]. The added thermal stress may have partially augmented the magnitude of the plasma concentrations observed. The increases observed in this study were even higher than a previous study examining β -endorphin/ β -lipotropin responses in humans to heat stress

[16]. Still, due to the higher thermal stress and exercise intensities used in the present study, our data appear to be consistent with these previous findings that peripheral plasma concentrations increase in response to increased exercise-thermal stress [16]. The lack of any significant bivariate relationships to core temperature changes or exercise intensity suggests that the response may be due to a combination of factors including both heat and exercise stress.

The increase in cortisol pre- to post-exercise was probably due to marked increases in the release of ACTH in response to the combined exercise and heat stress. The significant correlation between plasma levels of β -endorphin and cortisol are consistent with this hypothesis. Furthermore, exercise has been shown to stimulate concomitant ACTH and β -endorphin release [11,12].

No significant differences were observed for Peptide F ir in the heat. Still, it is interesting to note that both resting and post-exercise values are higher than previously reported [19]. We did observe significantly higher resting levels of Peptide F after 20 min of heat exposure compared to ambient conditions (25°C). The adrenal medullary chromaffin cells have been shown to be a major source of enkephalin-containing polypeptides secreted by the same stimuli which induces epinephrine release in these same cells [17, 22, 25]. Previous work has demonstrated that passive heating (50°C) may not increase epinephrine values [18]. Thus, higher resting levels of Peptide F ir observed at rest may be an acute response to heat exposure and require further study in its relationship to epinephrine release.

Administration of Met-enkephalin has been shown to effect body temperature [5]. Its effects are related to the dose and hypothesized to be a function of central mechanisms [5,10]. The lack of any changes pre- to post-exercise in this study disagrees with previously reported increases of Met-enkephalin ir following exercise [15]. This may be a function of degradation in the peripheral plasma [13]. Still, the values reported in this study are much lower than other data examining exercise responses of Met-enkephalin in thermoneutral environments [15]. The lower values of Met-enkephalin ir observed in this study may be in part due to the higher concentrations of precursor proenkephalin fragments in the heat. This possible mechanism is supported by the higher levels of Peptide F ir found both at rest and following exercise in the heat.

In summary, while the possible roles of EOP and thermal stress have been addressed before, little data were previously available which examined possible heat acclimation changes. Our data demonstrated that peripheral concentrations of different EOP do not reflect heat adaptations gained over an 8-day heat acclimation regime. Heat and exercise stress may result in a reduction of Met-enkephalin ir found in the peripheral plasma due to degradation or a decreased processing of larger precursor fragments. The different pattern of responses of β -endorphin compared to Met-enkephalin and Peptide F supports the possibility of separate physiological roles for these opioid peptides during exercise in the heat.

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